androst-5-en-17-one (U-14226 A).

The reaction mixture contained in the final volume of 1 mL: [^{3}H]squalene (10 000 cpm) diluted with squalene (final concentration = 20 μM), Tween-80 (final concentration = 0.05% w/v), 0.1 M K/K phosphate buffer pH 7.4 containing 1 mM EDTA, microsomes (5 mg of proteins) S_{105} (10 mg of proteins), U-14226A (50 μM), $NADP^+$ (2 mM), glucose-6-P (5 mM), glucose-6-P dehydrogenase (1 UI), $MgCl_2$-$6H_2O$ (5 mM).

Incubations lasted 30 min at 37 °C. The reaction was stopped by the addition of 1 mL of 10% ethanolic KOH and saponification for 30 min at 80 °C. Extraction and chromatographic procedures similar to those of the SO cyclase assay were used.

After developing the TLC in CH_2Cl_2, the areas corresponding to authentic squalene and SO were scraped and counted for radioactivity in a Beckman LS 5000 liquid scintillator. The enzymatic activity was expressed as nanomoles of SO formed/hour.

Acknowledgment. This work was supported by grants from the Ministero dell' Università e della Ricerca Scientifica e Tecnologica and from CNR Progetto di Chimica Fine, n. 91.01677.PF72. We thank the NMR group of the Dipartimento di Chimica Inorganica, Chimica Fisica e Chimica dei Materiali, Università di Torino; the mass group of the CNR, Area della Ricerca di Padova; and the mass group of the Dipartimento di Chimica Organica e Industriale, Università di Milano.

Novel Indolodioxanes with Antihypertensive Effects: Potent Ligands for the 5-HT_{1A} Receptor

Michael D. Ennis,*,† Mark E. Baze,† Martin W. Smith,‡ Charles F. Lawson,‡ Robert B. McCall,§ Robert A. Lahti,∥ and Montford F. Piercey∥

Departments of Medicinal Chemistry, Chemical and Biological Screening, Cardiovascular Diseases Research, and CNS Research, The Upjohn Company, Upjohn Laboratories, Kalamazoo, Michigan 49001. *Received May 13, 1991*

The synthesis and biological evaluation of a new family of tricyclic indolodioxanes is described. These compounds all contain the 2,3-dihydro-7*H*-1,4-dioxino[2,3-*e*]indole nucleus and bear substituents at the 2 and/or 8 positions. Thirteen members of this class were prepared and shown to be potent ligands for the 5-HT_{1A} receptor, with several compounds displaying subnanomolar inhibition constants. These compounds also bind to the dopamine D-2 receptor, but generally with higher inhibition constants than those for 5-HT_{1A}. Certain members of this novel structural class show in vivo activity in the mouse hypothermia assay. One of these compounds, U-86192A, has been shown to have antihypertensive effects in the cat, completely eliminating sympathetic nerve discharge at 1 mg/kg iv and lowering mean arterial pressure to 50% pretreatment levels. These effects can be reversed by the administration of spiperone, indicating that U-86192A is acting via a central serotonergic mechanism.

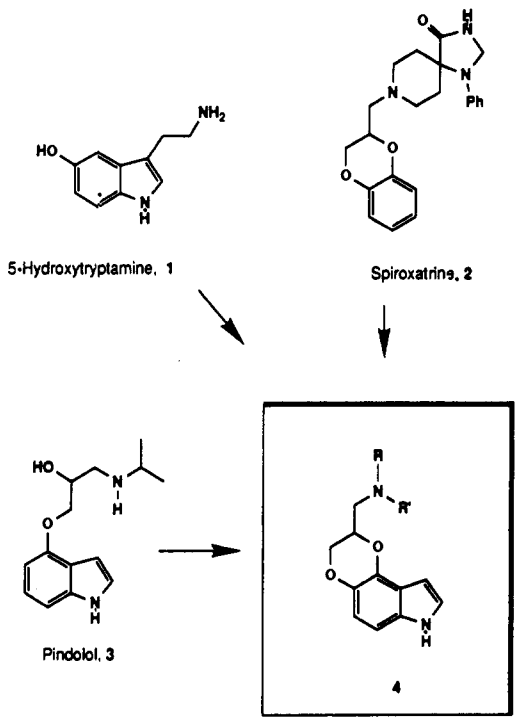
The neurotransmitter serotonin (1, 5-hydroxytryptamine, 5-HT) is associated with an ever-growing family of receptor subtypes.[1] One can comfortably reconcile the diversity of pharmacological events with which serotonin has been linked with the existence of these multiple binding sites. For example, there is strong evidence suggesting that activation of one such binding site, the 5-HT_{1A} receptor, inhibits sympathetic nerve discharge and might therefore play a central role in the regulation of blood pressure.[2] Critical to the study of any receptor-mediated event is the availability of chemical agents which bind with high selectivity to the receptor of interest. In this paper we will discuss the design rationale, the chemical synthesis, and the biological evaluation of a novel series of tricyclic indoles which possess remarkable binding affinities for the 5-HT_{1A} receptor. We will provide data which suggest that these compounds hold promise as novel, centrally-acting antihypertensive agents.

When considering structural skeleta as targets for centrally-acting cardiovascular agents, one can justifiably focus upon substituted 1,4-benzodioxanes as prime candidates. These compounds have a long history as antihypertensive agents which act primarily through adrenergic blockade.[3] Recently, however, it has become recognized that certain 1,4-benzodioxanes possess good affinity for the 5-HT_{1A} receptor. For example, the α_1-adrenergic agent WB 4101 binds to 5-HT_{1A} with an IC_{50} = 3.8 nM,[4] and the well-known 5-HT_{1A} antagonist spiroxatrine (2) displays a marked preference for the 5-HT_{1A} receptor over the 5-HT_{1B} or the 5-HT_2 sites.[5] Pharmacological activity believed to be mediated by 5-HT_{1A} receptors has been displayed by the 1,4-benzodioxanes (+)-flesinoxan and MDL 73005EF, the former possessing antihypertensive activity[6] and the latter active as an anxiolytic.[7] We have designed a series of compounds, illustrated generically as 4, which

† Department of Medicinal Chemistry.
‡ Department of Chemical and Biological Screening.
§ Department of Cardiovascular Diseases Research.
∥ Department of CNS Research.

(1) Leff, P.; Martin, G. R. The Classification of 5-Hydroxytryptamine Receptors. *Med. Chem. Rev.* **1988**, *8*, 187–202. Glennon, R. A. Central Serotonin Receptors as Targets for Drug Research. *J. Med. Chem.* **1987**, *30*, 1–12.
(2) Kuhn, D. M.; Wolf, W. A.; Lovenberg, W. Review of the Role of the Central Serotonergic Neuronal System in Blood Pressure Regulation. *Hypertension* **1980**, *2*, 243–255.
(3) Quaglia, W.; Pigini, M.; Giannella, M.; Melchiorre, C. 3-Phenyl Analogues of 2-[[[2-(2,6-Dimethoxy-Phenoxy)ethyl]-amino]-methyl]1,4-benzodioxan (WB 4101) as Highly Selective α_1-Adrenoreceptor Antagonists. *J. Med. Chem.* **1990**, *33*, 2946–2948.
(4) Norman, A. B.; Battaglia, G.; Morrow, A. L.; Creese, I. [^{3}H]-WB4101 labels S_1 serotonin receptors in rat cerebral cortex. *Eur. J. Pharmacol.* **1985**, *106*, 461–462.
(5) Nelson, D. L.; Taylor, E. W. Spiroxatrine: A Selective Serotonin$_{1A}$ Receptor Antagonist. *Eur. J. Pharm.* **1986**, *124*, 207–208.
(6) Wouters, W.; Tulp, M. Th. M.; Bevan, P. Flesinoxan lowers blood pressure and heart rate in cats via 5-HT_{1A} receptors. *Eur. J. Pharmacol.* **1988**, *149*, 213–223.
(7) Moser, P. C.; Tricklebank, M. D.; Middlemiss, D. N.; Mir, A. K.; Hibert, M. F.; Fozard, J. R. Characterization of MDL 73005EF as a 5-HT_{1A} selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. *Br. J. Pharmacol.* **1990**, *99*, 343–349.

0022-2623/92/1835-3058$03.00/0

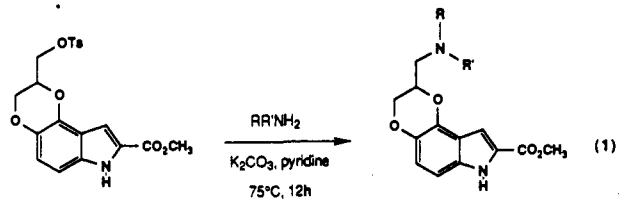


represent an amalgamation of the 1,4-benzodioxane structure of spiroxatrine with the indole nucleus of serotonin and the 5-HT_{1A} antagonist pindolol (3). The tricyclic indolodioxanes represented by 4 are virtually unknown in the scientific literature, making them attractive targets in their own right for synthesis and biological evaluation.

Although the hybrid molecule 4 incorporates structural elements of 1–3, it is unlikely that the binding interactions of 4 with the 5-HT_{1A} receptor parallel those of serotonin, spiroxatrine, or pindolol. Upon overlay of the indole nuclei of 1 and 4, it is difficult to coax the basic amines of these compounds into the same location. Furthermore, whereas the binding affinity of 4 appears to be fairly insensitive to substitution at the indole C-2 position (vide infra), the same is not true for serotonin.[8] Nevertheless, the indolodioxanes 4 represent a novel structural type possessing very high affinity for the 5-HT_{1A} receptor. We have demonstrated that one member of this new family, U-86192A, is a potent antihypertensive agent which is believed to be acting through a central serotonergic mechanism.

Chemistry

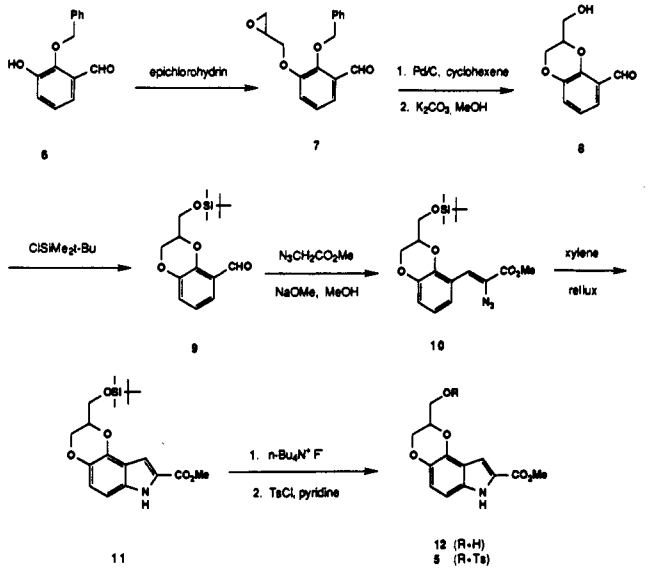
The majority of the compounds described in this study were prepared either directly or indirectly by the general reaction depicted in eq 1. Thus, the pivotal intermediate



5

5 became the first target for synthesis. The presence of the carbomethoxy group at the 2-position of the indole ring

Scheme I



is a consequence of our synthetic approach and could be either derivatized for analog generation or removed completely (vide infra). Outlined in Scheme I is our approach to 5. Alkylation of 2-(benzyloxy)-3-hydroxybenzaldehyde[9] with epichlorohydrin provided the epoxy aldehyde 7 in 96% yield. Catalytic hydrogenolysis removed the benzyl group and partially cyclized the intermediate phenol, a process which was driven to completion by brief treatment with triethylamine to afford an 80% yield of the 2,3-dihydro-8-formyl-2-(hydroxymethyl)-1,4-benzodioxin (8).[10] Although the subsequent indole-formation chemistry can be carried out with the primary hydroxyl of 8 left unprotected, we have found that higher yields and cleaner reactions are realized when this alcohol is first protected. Thus, 8 is converted into the corresponding *tert*-butyldimethylsilyl ether 9 in 84% yield under standard conditions. Condensation of 9 with α-azidoacetate[11] under carefully controlled conditions generates the vinyl azide 10, which upon thermolysis in refluxing xylene produces the indole 11 overall yields from 9 ranging from 65 to 75%.[12] Finally, desilylation of 11 with fluoride ion gives the alcohol 12, and subsequent tosylation affords the desired intermediate 5 in 79% overall yield.

The reaction depicted in eq 1 was most successful when cyclic nucleophiles such as piperidines were employed. Listed in Table I are the compounds prepared for this study. By means of the reaction illustrated in eq 1, com-

(8) Engel, G.; Göthert, M.; Hoyer, D.; Schlicker, E.; Hillenbrand, K. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *332*, 1–7.

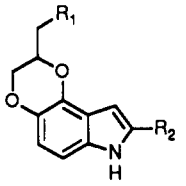
(9) 2-(Benzyloxy)-3-hydroxybenzaldehyde is prepared from 2,3-dihydroxybenzaldehyde in 62% yield following the literature reference: Kessar, S. V.; Gupta, Y. P.; Mohammad, T.; Goyal, M.; Sawall, K. K. Regioselective Mono-O-Alkylation of Some Pyrocatechoxide Dianions. *J. Chem. Soc., Chem. Commun.* **1983**, 400–401.

(10) It should be noted that the more straightforward approach of directly reacting 2,3-dihydroxybenzaldehyde with epichlorohydrin generates an inseparable mixture of 8 and the isomeric 2,3-dihydro-5-formyl-2-(hydroxymethyl)-1,4-benzodioxin. The structure of 8 was verified by X-ray crystallography.

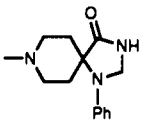
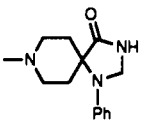
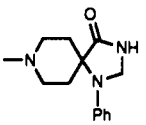
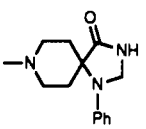
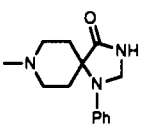
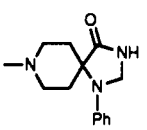
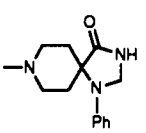
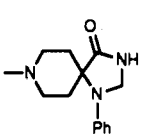
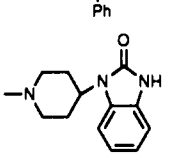
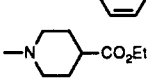
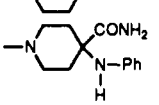
(11) Forster, M. O.; Fierz, H. E. The Triazo-Group. Part I. Triazoacetic Acid and Triazoacetone (Acetonylazoimide). *J. Chem. Soc.* **1908**, *93*, 72–85. Lieber, E.; Chao, T. S.; Rao, C. N. R. Improved Method for the Synthesis of Alkyl Azides. *J. Org. Chem.* **1957**, *22*, 238–240.

(12) Hemetsberger, H.; Knittle, D. Synthese und Thermolyse von α-Azidoacrylestern. *Monatsch. Chem.* **1972**, *103*, 194–204. Boger, D. L.; Coleman, R. S. Diels-Alder Reactions of Heterocyclic Azadienes: Total synthesis of PDEI, PDEII, and PDEI Dimer Methyl Ester. *J. Am. Chem. Soc.* **1987**, *109*, 2717–2727.

Table I. Indolodioxanes I



I

compd	R_1	R_2	formula	% yield[a]	mp, °C	analysis[b]
13		CO_2CH_3	$C_{26}H_{28}N_4O_5 \cdot {}^1/_2CH_3OH$	56		C, H, N
14		COOH	$C_{25}H_{26}N_4O_5$	78[c]	230 dec	C, H, N
15		$CONH_2$	$C_{25}H_{27}N_5O_4$	63[d]	250 dec	C, H, N
16		CO_2n-Bu	$C_{29}H_{34}N_4O_5$	76[e]	202.5–204.5	C, H, N
17		CO_2CH_2Ph	$C_{32}H_{32}N_4O_5$	61[e]	217.0–219.0	C, H, N
18		CN	$C_{25}H_{25}N_5O_4$	94[f]		HRMS[g]
19		CH_2OH	$C_{25}H_{28}N_4O_4$	16[h]	219.5–220.5	HRMS[g]
20		H	$C_{24}H_{26}N_3O_3$	*i*	228.0–230.0	C, H, N
21		CO_2CH_3	$C_{25}H_{26}N_4O_5 \cdot HCl$	45	300 dec	C, H, N
22		CO_2CH_3	$C_{21}H_{26}N_2O_6$	53	153.0–154.5	C, H, N
23		CO_2CH_3	$C_{25}H_{28}N_4O_5$	58	240.0–241.0	C, H, N
24[j]	$NH(CH_2)_3Ph$	CO_2CH_3	$C_{22}H_{24}N_2O_4 \cdot HCl \cdot {}^1/_2CH_3OH$	47[i]		C, H, N
25[j]	$NH(CH_2)_3Ph$	$CONH(CH_2)_3Ph$	$C_{30}H_{33}N_3O_3 \cdot HCl \cdot {}^1/_2CH_3OH$	44[i]		C, H, N

[a] Unless otherwise noted, prepared according to eq 1. [b] Analyses for the indicated elements were within ± 0.40% of the calculated values. [c] Prepared by hydrolysis of 13. [d] Prepared from 13 via ref 14. [e] Prepared by alcoholysis of 13 in the presence of 3-Å molecular sieves. [f] Prepared from 15 by dehydration with Burgess' reagent. [g] Satisfactory HRMS to within 0.004 mass units of the calculated value was obtained. [h] Isolated as a minor product from LAH reduction of 13. [i] See text. [j] Data obtained from the HCl salt.

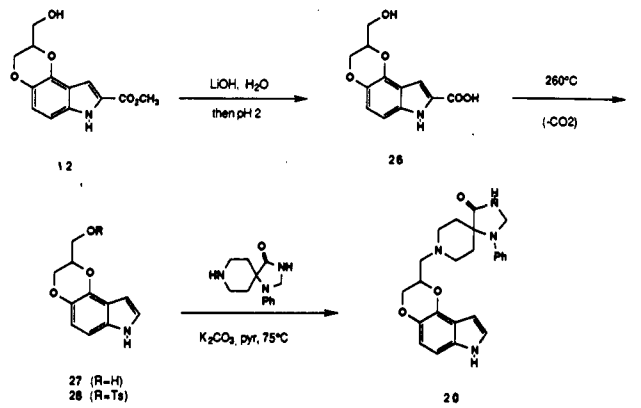
pounds 13, 21, 22, and 23 were prepared in yields ranging from 45% to 58%. For the primary amine adducts 24 and 25, 3-phenylpropylamine was employed in excess as the solvent and resulted in the production of nearly equal amounts of the two products. The ester 13 served as a common intermediate for several of the analogs in Table I. Compounds 14–17 and 19 were prepared from 13 by straightforward single-step reactions. The nitrile 18 was

Table II. Receptor Binding Data for Indolodioxanes and Selected Mouse Hypothermia Results

	receptor binding data: K_i, nM					mouse hypothermia	
compd	5-HT_{1A}	D-2	5-HT_2	α-1	opioid	dose, mg/kg (route)	max temp decrease, °F
13	0.9 ± 0.1	3.1 ± 0.2	640 ± 58	11.4 ± 1.6	6.5 ± 1.6	30 (sc)	3.2
14	17.8 ± 2.9	58 ± 20	>1722	>862	131 ± 64	17.3 (sc)	3.6
15	0.3 ± 0.1	1.8 ± 0.4	>1722	29.4 ± 15	0.6 ± 0.2		
16	3.0 ± 0.5	5.7 ± 1.5	92.6 ± 10.4	39.6 ± 3.9	3.1 ± 0.6		
17	1.0 ± 0.2	5.8 ± 1.8	143 ± 47	30.4 ± 2.9	14.4 ± 5.3	17.3 (sc)	5.6
18	0.5 ± 0.1	1.5 ± 0.2	281 ± 37	6.0 ± 0.3	0.1 ± 0.1	0.01 (sc)	13.0
						>30 (po)	
19	0.6 ± 0.1	2.0 ± 0.3	>1722	8.8 ± 2.2	3.3 ± 1.6		
20	0.4 ± 0.11	0.2 ± 0.05	ND[a]	ND	ND		
21	0.6 ± 0.1	6.7 ± 1.0	31.6 ± 2.8	1.8 ± 0.1	4.3 ± 1.3		
22	1.2 ± 0.2	255 ± 166	170 ± 19	12.3 ± 2.0	2425 ± 498	>30 (sc)	
23	22 ± 8.3	>388	171 ± 23	19.8 ± 5.9	3231 ± 620		
24	0.2 ± 0.1	15.9 ± 6.7	38.7 ± 3.6	0.6 ± 0.1	765 ± 147	1.3 (sc)	13.6
						1.7 (po)	8.8
25	1.6 ± 0.2	41 ± 22	27.6 ± 6.2	11.4 ± 2.9	530 ± 231	>30 (sc)	

[a] ND = not determined.

Scheme II



generated from the amide **15** by dehydration with Burgess' reagent.[13]

As previously stated, the functionality at the indole C-2 carbon was the result of our synthetic approach and not a natural consequence of our original design rationale. Thus, it seemed desirable to prepare at least one representative compound which bore only a hydrogen substituent at that position. The synthesis of such a molecule is illustrated in Scheme II. Hydrolysis of **12** gave the hydroxy acid **26** in 96% yield. This acid was subjected to thermal decarboxylation at 260 °C for 30 min to afford the desired product **27** in 62% isolated yield. Standard tosylation followed by nucleophilic displacement with the spiperone piperidine ultimately provided the representative 2-unsubstituted product **20** (48% overall from **27**).

Biological Results and Discussion

Inhibition constants for the binding of compounds **13–25** to the 5-HT_{1A}, dopamine D-2, 5-HT_2, α_1-adrenergic, and opioid receptors were determined and are reported in Table II. These compounds were also examined for their affinity to the α_2-adrenergic, β-adrenergic, benzodiazepine, and cholinergic receptors and found to be generally inactive.[15] As a class, the indolodioxanes **13–25** showed the greatest affinity for the 5-HT_{1A} site than for any other receptor examined, with several members displaying subnanomolar inhibition constants. It should be noted that none of the synthetic intermediates involved in the construction of these compounds possessed affinity for any receptor tested.

The first eight entries in Table II are compounds which all bear the spiperone–piperidine moiety as the benzodioxane substituent but vary the functionality at the indole C-2 position. From the superior binding affinities of nearly all these structures one can conclude that the region of the receptor pocket in which the C-2 substituent resides is neither very sterically nor electronically demanding. In contrast, however, it is known that 2-methylation of serotonin severely attenuates binding to the 5-HT_{1A} site.[8] The observation that the indolodioxanes accommodate a C-2 substituent as bulky as a butyl ester (**16**) argues against a commonality of binding orientation among the indole portions of serotonin and the compounds of this study. The relatively poor binding affinity for the C-2 carboxylic acid derivative **14** might indicate a preference for an uncharged functionality in this region of the receptor.[16]

Compounds **21–24** all retain a carbomethoxy group at the indole C-2 position but vary the benzodioxane amine substituent. As can be seen from Table II, the spiperone-piperidine is not required in order to achieve good binding. For example, compound **22**, derived from the simple 4-carbethoxypiperidine, was a very potent ligand for the 5-HT_{1A} receptor. Furthermore, we found that acyclic substituents could also give excellent binding results, as illustrated by the 3-phenylpropylamine derivatives **24** and **25**. Both of these compounds displayed inhibition constants for the 5-HT_{1A} receptor of less than 2 nM. It is interesting to note that the bis-adduct **25** still possesses excellent receptor affinity, once again demonstrating the structural promiscuity allowed at the indole C-2 position.

Of all the compounds in Table II, the worst ligand for the 5-HT_{1A} receptor is clearly compound **23**. This was most surprising, since this structure is so closely related

(13) Burgess, E. M.; Penton, H. R.; Taylor, E. A.; Williams, W. M. Conversion of Primary Alcohols to Urethanes via the Inner Salt of Methyl (carboxysulfamoyl)triethylammonium hydroxide: Methyl *n*-Hexylcarbamate. *Organic Syntheses*; Noland, W. E., Ed.; Wiley: New York, 1988; Collect. Vol. VI, p 788–791.

(14) Högberg, T.; Ström, P.; Ebner, M.; Rämsby, S. Cyanide as an Efficient and Mild Catalyst in the Aminolysis of Esters. *J. Org. Chem.* **1987**, *52*, 2033–2036.

(15) We define inactive as inhibiting the binding of test ligand at less than 50% at 1 μM concentration. The only activity found for these receptors was modest affinity of **18** for α_2-adrenergic sites (77% inhibition) and **25** for β-adrenergic (66% inhibition). There was good binding, however, of **24** for β-adrenergic (98% inhibition).

(16) The possibility that this region of the molecule does not intimately lie within the receptor pocket is discouraged by the observation that alkylation of the indole nitrogen results in a significant decrease in receptor affinity.

to 13, an excellent 5-HT_{1A} ligand. Remarkably, the excision of the methylene in the 5-membered ring of the spiropiperidine results in a greater than 100-fold decrease in binding affinity.

All of the indolodioxanes prepared in this study were also evaluated for their ability to bind to the dopamine D-2 site. In general, these compounds bind less well to the D-2 receptor than to the 5-HT_{1A} receptor. Only compound 20 displayed a preference for dopamine. Interestingly, this compound possesses a hydrogen as the indole C-2 substituent, suggesting perhaps that the dopamine receptor has a more severe steric requirement in this region than does the serotonin receptor. This notion receives support from the poor dopamine binding affinities of both 17 and 25. These two compounds bear bulky indole C-2 substituents (benzyl ester and 3-phenylpropylamide, respectively) and yet both have good affinity for 5-HT_{1A}.

The binding affinities for 13–25 at other central nervous system receptors were generally much weaker than those for the 5-HT_{1A} site. Notable exceptions were the potent α_1-adrenergic binding of 21 and 24 and the opioid affinities of 15, 16, 18, and 19. As stated earlier, adrenergic affinity is characteristic of 1,4-benzodioxanes and therefore it is not surprising to see this activity in our structural class. It has also been observed that the 4-anilinopiperidine moiety is a general pharmacophore for the opioid receptors.[17] The "spiperone–piperidine" portion of compounds 13–20 contain this pharmacophore (as does 21) and this might explain the affinities of these compounds for that receptor. Once again, the poor binding of 23 for the opioid receptor runs counter to this trend, although the very weak affinities for 22, 24, and 25 support these notions.

A select number of compounds were evaluated for in vivo effects in the mouse hypothermia assay (Table II). Compounds were chosen on the basis of their selectivity for the 5-HT_{1A} receptor over dopamine D-2, although some relatively nonselective examples were also run. Most of the compounds tested in this assay were only modestly active, but two compounds, 18 and 24, showed excellent potency. The extreme hypothermic response to 18 when administered subcutaneously is in marked contrast to the effect seen after oral administration. The in vivo pharmacology of 18 could be related to its high affinity for the dopamine receptor, which also promotes hypothermic activity. Compound 24, however, which displays a 20-fold preference for the 5-HT_{1A} receptor over D-2, also has good hypothermic activity, and additionally retains that activity upon oral administration.

From among all the compounds listed in Table II has emerged the 3-phenylpropylamine derivative 24 as the best candidate for further development. This compound, known also as U-86192A, possesses a very high affinity for the 5-HT_{1A} receptor and good selectivity over dopamine. Excellent oral bioavailability is indicated by the in vivo mouse hypothermia assay. We therefore decided to further examine this compound for cardiovascular activity. Shown in Figure 1 are the effects of U-86192A on mean arterial pressure, heart rate, and sympathetic nerve discharge (SND) upon intravenous administration in the intact cat. At 1 mg/kg iv, U-86192A reduced blood pressure and to nearly 50% of pretreatment levels while nearly completely shutting off sympathetic nerve discharge. Indeed, the dose–response curve for inhibition of SND by U-86192A closely follows that of 8-OH-DPAT. The effects of U-86192A on heart rate also paralleled that of 8-OH-DPAT.

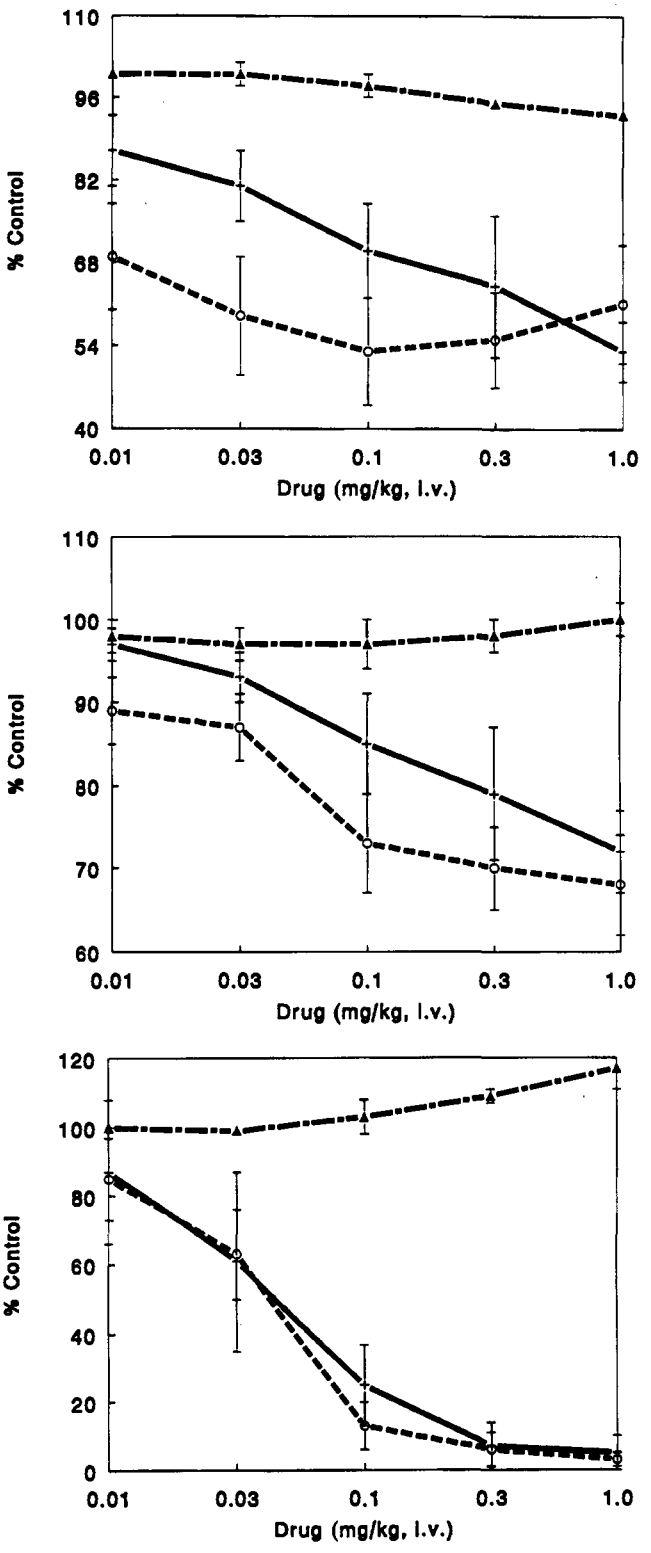


Figure 1. Effects of U-86192A on mean arterial blood pressure (top), heart rate (middle), and sympathetic nerve discharge (bottom): +, U-86192, o, 8-OH-DPAT, ▲, saline.

Although the binding selectivity of U-86192A for the 5-HT_{1A} receptor is generally good, we recognized that its strong affinity for the α_1-adrenergic site might be responsible for the in vivo cardiovascular effects observed. To address this concern, we demonstrated that the reduction of sympathetic nerve discharge by U-86192A could be reversed upon treatment with the 5-HT_{1A} antagonist spiperone. Following administration of U-86192A and subsequent elimination of SND activity, treatment with spiperone (1 mg/kg) caused a return of nerve activity to

(17) Lenz, G. R.; Evans, S. M.; Walters, D. E.; Hopfinger, A. J. *Opiates*; Academic Press: New York, 1986; p 318.

64% of pretreatment levels. It has previously been shown that spiperone will reverse the effects of 8-OH-DPAT in a similar manner.[18] Thus, we feel these results suggest that the antihypertensive activity of U-86192A is mediated by the 5-HT_{1A} receptor.

Conclusion

We have described the synthesis and preliminary biological properties of a novel class of indolodioxanes. This family of compounds is generally characterized by remarkably high binding affinities for the 5-HT_{1A} receptor. Many members of this exciting new family class of compounds possess in vivo biological activity. One such compound, U-86192A, has excellent oral availability and is an effective antihypertensive agent in the cat. These compounds, and others like them, represent a novel structural class of 5-HT_{1A} agonists and provide new leads for pursuing the control of blood pressure through centrally-acting agents.

Experimental Section

Chemistry. Proton and carbon magnetic resonance spectra were recorded on a Bruker Aspect 3000 spectrometer and are reported in ppm on the δ scale from internal tetramethylsilane. Infrared spectra were obtained using a Digilab Model FTS-40 spectrophotometer. Mass spectra were obtained with a Varian MAT CH5-DF spectrometer and are reported as $[M + H]^+$ ions. Elemental analyses were determined by the Physical and Analytical Chemistry Department (The Upjohn Laboratories). Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values. Melting points were determined on either a Thomas-Hoover capillary melting point apparatus or a Mettler FP62 and are uncorrected.

Unless otherwise noted, all nonaqueous reactions were carried out under an inert atmosphere using oven-dried glassware. Anhydrous tetrahydrofuran refers to material that was distilled from sodium metal/benzophenone ketyl. Dichloromethane, pyridine, and benzene were dried over activated 4-Å molecular sieves. Thin-layer chromatography was performed using Analtech 250-μm silica gel GF plates. Flash chromatography was carried out on EM Reagents silica gel 60 (230–400 mesh).

3-(Oxiranylmethoxy)-2-(phenylmethoxy)benzaldehyde (7). A solution of 2-(benzyloxy)-3-hydroxybenzaldehyde[9] (26.78 g, 0.117 mol) in absolute ethanol (120 mL) was treated with 1.0 N aqueous sodium hydroxide (117 mL, 0.117 mol) and briefly heated to reflux under nitrogen. The black solution was cooled to room temperature and epichlorohydrin (92.6 mL, 1.16 mol, 10 equiv) was added in a single portion. The solution was brought to reflux using a preheated oil bath (110 °C) and was heated under nitrogen a total of 30 min. After cooling to room temperature, the ethanol was removed in vacuo and the aqueous remainder was diluted with water (650 mL) and extracted with ethyl acetate (3 × 350 mL). The combined organic layers were washed once with brine (150 mL) and dried over anhydrous magnesium sulfate. After filtration and concentration in vacuo, the residue was passed through a short column of silica gel using 40% ethyl acetate/hexane to remove polar, yellow material. The product thus obtained was recrystallized from ether to give 26.12 g (78%) of 7 as a white solid, mp 62–63 °C. The mother liquor was concentrated and chromatographed on 600 g of silica gel using 20% ethyl acetate/hexane to give an additional 5.93 g of 7 as a white solid (total yield 96%): NMR ($CDCl_3$) δ 10.24 (s, 1 H, CHO), 7.5–7.1 (m, 8 H, aromatic H's), 5.21 (s, 2 H, $PhCH_2$), 4.39 (dd, J = 11.1, 2.8 Hz, 1 H, $ArOCH_{2a}$), 4.03 (dd, J = 11.1, 5.9 Hz, 1 H, $ArOCH_{2b}$), 3.43 (m, 1 H, OCH), 2.94 (t, J = 4.6 Hz, 1 H, OCH_{2a}), 2.80 (dd, J = 4.9, 2.6 Hz, 1 H, OCH_{2b}). Anal. ($C_{17}H_{16}O_4$) C, H.

2,3-Dihydro-3-(hydroxymethyl)-1,4-benzodioxin-5-carboxaldehyde (8). A solution of 7 (14.22 g, 50 mmol), cyclohexene (20.3 mL, 0.2 mol), and 10% palladium on carbon (1.40 g) in ethyl acetate (500 mL) was heated to reflux for 21 h. After cooling to room temperature, the mixture was filtered through Celite and concentrated in vacuo. The resulting residue was dissolved in ethanol (200 mL) and treated with triethylamine (14 mL, 0.10 mol) and water (200 mL). The solution was refluxed under nitrogen for 1 h and then cooled to room temperature and concentrated in vacuo at 40 °C on the rotary evaporator. The yellow residue thus obtained was chromatographed on 500 g of silica gel with 40% ethyl acetate/hexane to give 7.77 g (80%) of 8 as an off-white solid. Recrystallization from ethyl acetate/hexane provided an analytical sample as off-white, nondescript crystals: mp 70–71.5 °C; NMR ($CDCl_3$) 10.32 (s, 1 H, CHO), 7.38 (dd, J = 7.7, 1.6 Hz, 1 H, aromatic H), 7.11 (dd, J = 8.0, 1.6 Hz, 1 H, aromatic H), 6.92 (t, J = 7.8 Hz, 1 H, aromatic H), 4.37 (m, 2 H, OCH and $ArOCH_{2a}$), 4.20 (dd, J = 12.0, 8.2 Hz, 1 H, $ArOCH_{2b}$), 4.00 (dd, J = 12.2, 4.0 Hz, 1 H, OCH_{2a}), 3.92 (dd, J = 12.2, 4.5 Hz, 1 H, OCH_{2b}), 3.31 (broad s, 1 H, OH). Anal. ($C_{10}H_{10}O_4$) C, H.

3-[(*tert*-Butyldimethylsiloxy)methyl]-2,3-dihydro-1,4-benzodioxin-5-carboxaldehyde (9). 4-(Dimethylamino)pyridine (0.79 g, 6.50 mmol) was added in a single portion to a solution of 8 (971 mg, 5.00 mmol) and *tert*-butyldimethylsilyl chloride (0.90 g, 6.00 mmol) in dry dichloromethane (10 mL) at 0 °C. The cooling bath was removed and the solution was allowed to stir overnight at room temperature. The reaction mixture was then diluted with dichloromethane (100 mL), washed with water (50 mL) and saturated aqueous ammonium chloride (50 mL), and then dried over anhydrous sodium sulfate. After filtration and concentration in vacuo, the resulting residue was chromatographed on 50 g of silica gel using 5% ethyl acetate/hexane to give 1.30 g (84%) of 9 as a colorless syrup which solidified on refrigeration: mp 32–33 °C; NMR ($CDCl_3$) δ 10.42 (d, J = 0.5 Hz, 1 H, CHO), 7.41 (dd, J = 7.7, 1.6 Hz, 1 H, aromatic H), 7.11 (dd, J = 8.0, 1.7 Hz, 1 H, aromatic H), 6.90 (dt, J = 7.8, 0.6 Hz, 1 H, aromatic H), 4.4–4.3 (m, 2 H, OCH_{2a} and OCH), 4.17 (dd, J = 11.1, 6.6 Hz, 1 H, OCH_{2b}), 3.96 (dd, J = 10.7, 4.4 Hz, 1 H, $SiOCH_{2a}$), 3.87 (dd, J = 10.7, 6.2 Hz, 1 H, $SiOCH_{2b}$), 0.90 (s, 9 H, CCH_3's), 0.10 (s, 6 H, $SiCH_3$'s). Anal. ($C_{16}H_{24}O_4Si$) C, H.

Methyl 2-Azido-3-[3-[(*tert*-butyldimethylsiloxy)methyl]-2,3-dihydro-1,4-benzodioxin-5-yl]propenoate (10). A solution of sodium methoxide in methanol (25 wt %, 19.1 mL, 83 mmol) was added dropwise over 5 min to a solution of 9 (3.21 g, 10.4 mmol) and methyl azidoacetate (11.97 g, 104 mmol) in dry methanol (25 mL) at −22 °C. The temperature was raised to −5 °C and stirring was continued for 30 min, at which point additional methanol (10 mL, precooled) was added to thin the reaction mixture. After stirring overnight at −5 °C, the dark reaction mixture was poured into ice-cold saturated aqueous ammonium chloride (110 mL) and extracted with ice-cold ethyl acetate (3 × 110 mL). The combined organic phases were washed with ice-cold brine (1 × 55 mL) and dried over anhydrous sodium sulfate. After filtration and concentration in vacuo, the resulting residue (minus 8% removed for exploratory work) was chromatographed on 300 g of silica gel using 2.5% ethyl acetate/hexane to give 2.81 g (72%) of 10 as a yellow oil: NMR ($CDCl_3$) δ 7.80 (dd, J = 6.4, 3.2 Hz, 1 H, aromatic H), 7.32 (s, 1 H, vinylic H), 6.87 (m, 2 H, aromatic H's), 4.3–4.2 (m, 2 H, OCH_{2a} and OCH), 4.09 (dd, J = 10.9, 6.5 Hz, 1 H, OCH_{2b}), 3.95–3.8 (m, 2 H, $SiOCH_{2a}$ and $SiOCH_{2b}$), 3.90 (s, 3 H, OCH_3), 0.90 (s, 9 H, CCH_3's), 0.09 (s, 6 H, $SiCH_3$'s); HRMS, m/e 405.1713 ($C_{16}H_{27}N_3O_5Si$ requires 405.1720).

2,3-Dihydroxy-2-[(*tert*-butyldimethylsiloxy)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Methyl Ester (11). A solution of 10 (16.5 g, 40.7 mmol) in *o*-xylene was refluxed (oil bath preheated to 180 °C) for 1.5 h. The solvent was removed in vacuo at 60 °C to give a yellow solid residue which was recrystallized from hexane (ca. 200 mL) to give 11.6 g (75%) of 11 as fine, white needles: mp 141.5–142.5 °C; R_f 0.16 (10% ethyl acetate/hexane); NMR ($CDCl_3$) δ 9.11 (broad s, 1 H, NH), 7.23 (d, J = 1.9 Hz, 1 H, vinylic H), 6.94 (d, J = 8.8 Hz, 1 H, aromatic H), 6.88 (d, J = 8.8 Hz, 1 H, aromatic H), 4.4–4.3 (m, 2 H, OCH and OCH_{2a}), 4.13 (dd, J = 11.6, 7.1 Hz, 1 H, OCH_{2b}), 4.05–3.85 (m, 2 H, $SiOCH_{2a}$ and $SiOCH_{2b}$), 3.94 (s, 3 H, OCH_3), 0.91 (s, 9 H, CCH_3's), 0.12 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$). Anal. ($C_{19}H_{27}NO_5Si$) C, H, N.

(18) Lum, J. A.; Piercey, M. F. Electrophysiological evidence that spiperone is an antagonist of 5-HT_{1A} receptors in the dorsal raphe nucleus. *Eur. J. Pharmacol.* **1988**, *149*, 9–15. Clement, M. E.; McCall, R. B. Studies on the site and mechanism of the sympatholytic action of 8-OH DPAT. *Brain Res.* **1990**, *525*, 232–241.

2,3-Dihydro-2-(hydroxymethyl)-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Methyl Ester (12). A solution of 11 (3.78 g, 10.0 mmol) in dry tetrahydrofuran (35 mL) was treated with 1 M tetra-*n*-butylammonium fluoride in tetrahydrofuran (11.0 mL, 11.0 mmol) at room temperature. After stirring for 1 h and 20 min, the mixture was poured into saturated aqueous ammonium chloride (115 mL). The organic solvents were removed in vacuo and the aqueous remainder was further diluted with water and then extracted with ethyl acetate (3 × 70 mL). The combined organic layers were washed with brine (25 mL) and then dried over anhydrous magnesium sulfate. After filtration and concentration, the resulting residue was chromatographed on 70 g of silica gel using 40% ethyl acetate/hexane until 12 began to elute, at which point the eluant was changed to 75% ethyl acetate/hexane. Thus was obtained 2.60 g (99%) of 12 as a white solid: mp 158–160 °C (from ethyl acetate/hexane); NMR ($CDCl_3$ + few drops CD_3CN) δ 9.07 (broad s, 1 H, NH), 7.24 (d, J = 7.24 Hz, 1 H, vinylic H), 6.91 (s, 2 H, aromatic H's), 4.45–4.3 (m, 2 H, OCH and OCH_{2a}), 4.15 (dd, J = 11.2, 7.0 Hz, 1 H, OCH_{2b}), 3.92 (m and s, 5 H, OCH_3 and OCH_2), 1.86 (v broad s, 1 H, OH). Anal. ($C_{13}H_{13}NO_5$) C, H.

2,3-Dihydro-2-[[(*p*-tolylsulfonyl)oxy]methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Methyl Ester (5). *p*-Toluenesulfonyl chloride (660 mg, 3.46 mmol) was added in a single portion to a solution of 12 (759 mg, 2.88 mmol) and 4-(dimethylamino)pyridine (457 mg, 3.74 mmol) in dry dichloromethane at 0 °C. The cooling bath was removed and the solution was stirred overnight at room temperature. The white solid present after this time was collected and washed with a minimum amount of dichloromethane to give 960 mg (80%) of 5, mp 204–206 °C (ethyl acetate/hexane): R_f 0.33 (40% ethyl acetate/hexane); IR (mull) 3339, 2926, 1677, 1530, 1446, 1372, 1259, 1232, 1180, 774 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 11.88 (broad s, 1 H, NH), 7.80 (d, J = 8.2 Hz, 2 H, *m*-tosyl H's), 7.40 (d, J = 8.1 Hz, 2 H, *o*-tosyl H's), 6.92 (d, J = 8.8 Hz, 1 H, aromatic H), 6.82 (m, 2 H, aromatic H and vinylic H), 4.56 (m, 1 H, OCH), 4.43 (dd, J = 11.3, 2.9 Hz, 1 H, OCH_{2a}), 4.30 (m, 2 H, $SOCH_{2a}$ and $SOCH_{2b}$), 4.02 (dd, J = 11.5, 6.4 Hz, 1 H, OCH_{2b}), 3.89 (s, 3 H, OCH_3), 2.40 (s, 3 H, CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) δ 163.2, 146.8, 136.3, 136.0, 135.7, 133.6, 131.8, 129.4, 128.4, 119.6, 118.3, 107.2, 105.5, 72.4, 70.2, 65.4, 53.5, 22.8. Anal. ($C_{20}H_{19}NO_7S$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Methyl Ester (13). A mixture of 5 (3.17 g, 7.60 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (5.27 g, 22.8 mmol), and powdered potassium carbonate (5.25 g, 38.0 mmol) in dry pyridine (75 mL) was heated at 75 °C for 24 h. After cooling to room temperature, the black mixture was diluted with dichloromethane and filtered through Celite. The residue obtained on concentration of the filtrate was dissolved in a large volume of dichloromethane and chromatographed on 300 g of silica gel using 75% ethyl acetate/hexane to give 2.01 g (56%) of 13 as a pale yellow solid. Recrystallization from methanol gave an off-white solid: R_f 0.18 (75% ethyl acetate/hexane); IR (mull) 3320, 2954, 2924, 2856, 1714, 1690, 1529, 1259, 1237, 1217 cm^{-1}; NMR (DMSO-d_6) δ 11.85 (broad s, 1 H, indole NH), 8.66 (broad s, 1 H, lactam NH), 7.25 (t, J = 7.6 Hz, 2 H, *m*-phenyl H's), 7.00 (d, J = 2.0 Hz, 1 H, vinylic H), 6.95 (m, 4 H, *o*-phenyl H's and aromatic H's), 4.58 (s, 2 H, NCH_2N), 4.49 (m, 1 H, OCH), 4.36 (m, 1 H, OCH_{2a}), 4.05 (dd, J = 11.5, 6.7 Hz, 1 H, OCH_{2b}), 3.85 (s, 3 H, OCH_3), 3.0–2.5 (m, 8 H, NCH_2's and $NCCH_{2a}$'s), 1.60 (m, 2 H, $NCCH_{2b}$'s); ^{13}C NMR (300 MHz, DMSO-d_6) δ 177.2, 162.4, 144.3, 135.9 (overlap), 134.8, 129.9, 127.5, 119.1, 118.5, 117.6, 115.1, 106.0, 104.6, 72.5, 67.2, 59.6, 58.8, 58.5, 52.6, 51.4, 50.5 (differentiation of the piperidine-ring carbons α to the nitrogen), 29.4. Anal. ($C_{28}H_{28}N_4O_5 \cdot {}^1/_2CH_3OH$) C, H, N.

Compounds 21–23 were prepared by the procedure described above using the appropriate amine nucleophile.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid (14). A suspension of 13 (200 mg, 0.40 mmol) in methanol (2 mL) was treated with a solution of lithium hydroxide monohydrate (34 mg, 0.80 mmol) in water (1 mL). The heterogeneous mixture was heated under nitrogen at 60 °C for 4.5 h, during which time a clear amber solution gradually obtained. After cooling to room temperature, the solution was diluted with water (6 mL) and acidified to pH 7 with aqueous 1 N hydrochloric acid. The voluminous white solid which precipitated was filtered and purified by reprecipitation: a suspension of 14 in water was treated with aqueous 1 N sodium hydroxide until a clear solution obtained (pH 12), and then the pH was adjusted to 7 with aqueous 1 N hydrochloric acid. The resulting solid was filtered, washed with water, and air-dried on the filter funnel to give 145 mg (78%) of 14 as a beige powder (decomposes at ca. 230 °C with gas evolution): R_f 0.20 (100:50:5 chloroform/methanol/concentrated aqueous ammonium hydroxide). Anal. ($C_{25}H_{26}N_4O_5 \cdot 1.25H_2O$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic (15). A suspension of 13 (1.00 g, 1.92 mmol) and sodium cyanide (10 mg, 0.20 mmol) in 16% methanolic ammonia (100 mL) was heated in a pressure tube (Ace #15 thread) for 5 days at 100 °C, at which point the dark, homogeneous mixture was cooled and concentrated in vacuo. The residue was dissolved in dichloromethane/methanol and chromatographed on 50 g of silica gel using 75% ethyl acetate/hexane until a small amount of unreacted 13 was recovered. The chromatography solvent was then switched to 2% methanol/ethyl acetate to elute the product. Recrystallization from methanol gave two crops of 15 (558 mg, 63%) as a white solid: R_f 0.16 (1% methanol/ethyl acetate). Anal. ($C_{25}H_{27}N_5O_4 \cdot 0.5EtOAc$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, *n*-Butyl Ester (16). A suspension of 13 (95 mg, 0.200 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (3 mg, 0.020 mmol) in dry 1-butanol (8 mL) was refluxed under nitrogen for 31 h using a Soxhlet extractor containing 3-Å molecular sieves (1.8 g). The butanol was removed in vacuo and the solid residue was dissolved in dichloromethane (ca. 15 mL) and chromatographed on 10 g of silica gel using 75% ethyl acetate/hexane to give a white solid (105 mg). The solid was recrystallized from ethyl acetate to give 79 mg (76%) of 17 as very fine, white needles: mp 203.5–204.5 °C; R_f 0.21 (75% ethyl acetate/hexane). Anal. ($C_{29}H_{34}N_4O_5$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Benzyl Ester (17). A suspension of 13 (95 mg, 0.200 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (3 mg, 0.020 mmol) in dry benzyl alcohol (2 mL) and toluene (7 mL) was refluxed under nitrogen overnight using a Soxhlet extractor containing 3-Å molecular sieves (1.8 g). The toluene and the benzyl alcohol were removed in vacuo (the alcohol required Kugelrohr distillation at 90 °C/0.1 mmHg), and the residue, applied in dichloromethane, was chromatographed on 10 g of silica gel using 75% ethyl acetate/hexane to give a white solid (105 mg). The solid was recrystallized from acetone to give 68 mg (61%) of 18 as very fine, white needles: mp 217–219 °C; R_f 0.19 (75% ethyl acetate/hexane). Anal. ($C_{32}H_{32}N_4O_5$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carbonitrile (18). A solution of 15 (61 mg, 0.121 mmol) in dry tetrahydrofuran (4 mL) under nitrogen at room temperature was treated with the inner salt of [(methoxycarbonyl)sulfamoyl]triethylammonium hydroxide (Burgess' reagent) (3.2 mg, 0.133 mmol). After stirring for 1 h, a second portion of Burgess' reagent (32 mg, 0.133 mmol) was added and stirring was continued for an additional hour. The mixture was concentrated in vacuo and the residue was chromatographed on 5 g of silica gel using 3.5% methanol/dichloromethane to give 51 mg (94%) of 18 as a white solid: R_f 0.35 (5% methanol/dichloromethane); HRMS, m/e 443.1964 ($C_{25}H_{25}N_5O_3$ requires 443.1957).

2,3-Dihydro-2-(hydroxymethyl)-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid (26). A solution of lithium hydroxide monohydrate (190 mg, 4.52 mmol) in water (7 mL) was added to a solution of 12 (595 mg, 2.26 mmol) in methanol (14 mL) under nitrogen and the solution was heated at 60 °C for 1 h. The methanol was removed in vacuo and additional water (20 mL) was added to the aqueous remainder. The pH was adjusted to 2 with 1 N hydrochloric acid and the resulting thick, white precipitate was filtered and washed well with water. After air-drying for some time, further drying in vacuo gave 540 mg (96%) of 26 as a white powder. Recrystallization from ethyl acetate/ethanol/hexane gave an amorphous white solid: NMR (DMSO-d_6)

δ 11.65 (broad s, 1 H, NH), 6.94 (d, J = 1.8 Hz, 1 H, vinylic H), 6.89 (d, J = 8.9 Hz, 1 H, aromatic H), 6.83 (d, J = 8.8 Hz, 1 H, aromatic H), 4.29 (m, 2 H, OCH and $ArOCH_{2a}$), 4.02 (dd, J = 11.3, 6.9 Hz, 1 H, $ArOCH_{2b}$), 3.69 (m, 2 H, OCH_2). Anal. ($C_{12}H_{11}O_5N$) C, H, N.

2,3-Dihydro-2-(hydroxymethyl)-7*H*-1,4-dioxino[2,3-*e*]indole (27). A round-bottom flask containing solid **26** (407 mg, 1.63 mmol) under nitrogen was lowered into an oil bath preheated to 240 °C. The temperature was raised to 257 °C and maintained there for 30 min, during which time gas evolution from the now-liquid **26** occurred. After cooling to room temperature, the resulting residue was dissolved in 75% ethyl acetate/hexane and chromatographed on 40 g of silica gel using 40% ethyl acetate/hexane to give 208 mg (62%) of **27** as a near colorless syrup: NMR ($CDCl_3$) δ 8.14 (broad s, 1 H, NH), 7.09 (t, J = 2.8 Hz, 1 H, vinylic H), 6.87 (dd, J = 8.7, 0.9 Hz, 1 H, aromatic H), 6.79 (d, J = 8.7 Hz, 1 H, aromatic H), 6.56 (m, 1 H, vinylic H), 4.37 (m, 1 H, OCH), 4.30 (dd, J = 11.4, 2.3 Hz, 1 H, $ArOCH_{2a}$), 4.14 (dd, J = 11.4, 7.1 Hz, 1 H, $ArOCH_{2b}$), 3.92 (m, 2 H, OCH_2), 2.25 (broad s, 1 H, OH); HRMS, m/e 205.0745 ($C_{11}H_{11}NO_3$ requires 205.0739).

2,3-Dihydro-2-[[(*p*-tolylsulfonyl)oxy]methyl]-7*H*-1,4-dioxino[2,3-*e*]indole (28). *p*-Toluenesulfonyl chloride (212 mg, 1.11 mmol) was added in a single portion to a solution of **27** (190 mg, 0.926 mmol) and 4-(dimethylamino) pyridine (147 mg, 1.20 mmol) in dry dichloromethane (9 mL) at 0 °C. The cooling bath was removed and the solution was stirred at room temperature for 23 h. The reaction mixture was then sequentially washed with water (1 × 5 mL), saturated aqueous copper sulfate (2 × 5 mL), and water again (1 × 5 mL), and then dried over anhydrous sodium sulfate. After filtration and concentration in vacuo, the resulting solid was chromatographed on 20 g of silica gel using 25–30% ethyl acetate/hexane. The syrup initially obtained was dissolved in 60% ethyl acetate/hexane and allowed to stand, where upon 200 mg of **28** was deposited as a white, crystalline solid, mp 145–145.5 °C. The essentially pure mother liquor provided an additional 90 mg (total yield 87%): NMR (acetone-d_6) δ 10.18 (broad s, 1 H, NH), 7.82 (d, J = 8.3 Hz, 2 H, *m*-tosyl H's), 7.39 (d, J = 8.1 Hz, 2 H, *o*-tosyl H's), 7.23 (t, J = 2.8 Hz, 1 H, vinylic H), 6.91 (d, J = 8.8 Hz, 1 H, aromatic H), 6.63 (d, J = 8.7 Hz, 1 H, aromatic H), 6.36 (m, 1 H, vinylic H), 4.55 (m, 1 H, OCH), 4.45–4.2 (m, 3 H, $SOCH_{2a}$, $SOCH_{2b}$, and OCH_{2a}), 4.07 (dd, J = 11.5, 6.1 Hz, 1 H, OCH_{2b}), 2.41 (s, 3 H, CH_3). Anal. ($C_{16}H_{17}NO_5S$) C, H, N.

2,3-Dihydro-2-[(4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]-dec-8-yl)methyl]-7*H*-1,4-dioxino[2,3-*e*]indole (20). A solution of **28** (237 mg, 0.659 mmol), 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (458 mg, 1.98 mmol), and powdered, anhydrous potassium carbonate (456 mg, 3.30 mmol) in dry pyridine (7 mL) was heated to 75 °C overnight. The resulting black reaction mixture was diluted with dichloromethane and filtered through Celite. The filtrate was concentrated to near dryness in vacuo and the residue thus obtained was dissolved in a large volume of dichloromethane and chromatographed on 20 g of silica gel using 75% ethyl acetate/hexane to give 152 mg (55%) of **20** as a pale yellow solid. Recrystallization from ethyl acetate/ethanol/hexane gave a tan solid: mp 228–230 °C dec; NMR (DMSO-d_6) δ 10.93 (broad s, 1 H, indole NH), 8.65 (broad s, 1 H, lactam NH), 7.25 (t, J = 7.7 Hz, 2 H, *m*-phenyl H's), 7.19 (t, J = 2.7 Hz, 1 H, vinylic H), 6.86 (m, 3 H, *o*-phenyl H's and aromatic H), 6.76 (t, J = 7.3 Hz, 1 H, *p*-phenyl H), 6.65 (d, J = 8.6 Hz, 1 H, aromatic H), 6.31 (m, 1 H, vinylic H), 4.58 (s, 2 H, NCH_2N), 4.43 (m, 1 H, OCH), 4.34 (m, 1 H, OCH_{2a}), 4.02 (dd, J = 11.3, 6.7 Hz, 1 H, OCH_{2b}), 3.0–2.5 (m, 8 H, NCH_2's and $NCCH_{2a}$'s), 1.57 (m, 2 H, $NCCH_{2b}$'s). Anal. ($C_{24}H_{26}N_4O_3$) C, H, N.

2,3-Dihydro-2-[[(3-phenylpropyl)amino]methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxylic Acid, Methyl Ester (24) and 2,3-Dihydro-*N*-(3-phenylpropyl)-2-[[(3-phenylpropyl)-amino]methyl]-7*H*-1,4-dioxino[2,3-*e*]indole-8-carboxamide (25). A solution of **5** (162 mg, 0.388 mmol) and potassium carbonate (107 mg, 0.776 mmol) in 3-phenyl-1-propylamine (1.0 mL) was stirred at 75 °C for 5 h, resulting in formation of a thick gel. After cooling to room temperature, the mixture was diluted with water (25 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic layers were washed with water (5 mL) and dried over anhydrous sodium sulfate. After concentration in vacuo to remove the dichloromethane, the excess amine was removed be molecular distillation (Kugelrohr, bp 80 °C, 0.1 mm). The residue was chromatographed on 20 g of silica gel using 40–75% ethyl acetate/hexane to give 70 mg (47%) of **24** as a white solid, and 82 mg (44%) of **25** as a white, waxy solid. The hydrochloride salts were prepared by treatment of the free bases in ethyl acetate with methanolic hydrogen chloride. Recrystallization from methanol/ethyl acetate gave amorphous, white solids (melting points indefinite). For **24** (free base): NMR ($CDCl_3$) δ 9.31 (broad s, 1 H, indole NH), 7.22 (m, 6 H, phenyl H's and vinylic H), 6.92 (d, J = 8.8 Hz, 1 H, aromatic H), 6.88 (d, J = 8.8 Hz, 1 H, aromatic H), 4.42 (m, 1 H, OCH), 4.30 (dd, J = 11.4, 2.2 Hz, 1 H, OCH_{2a}), 4.06 (dd, J = 11.4, 7.0 Hz, 1 H, OCH_{2b}), 3.92 (s, 3 H, OCH_3), 2.99 (dd, J = 12.6, 7.0 Hz, 1 H, $NCH_{2a}CO$), 2.90 (dd, J = 12.6, 4.6 Hz, 1 H, $NCH_{2b}CO$), 2.69 (m, 4 H, NCH_2 and $PhCH_2$), 1.85 (quint, J = 7.6 Hz, 2 H, CH_2), 1.63 (broad s, 1 H, NH). Anal. ($C_{22}H_{24}N_2O_4 \cdot HCl \cdot {}^1/_2CH_3OH$) C, H, N. For **25** (free base): NMR ($CDCl_3$) 10.10 (broad s, 1 H, indole NH), 7.4–7.1 (m, 10 H, phenyl H's), 6.89 (d, J = 8.8 Hz, 1 H, aromatic H), 6.85 (d, J = 8.8 Hz, 1 H, aromatic H), 6.75 (d, J = 1.7 Hz, 1 H, vinylic H), 6.24 (broad t, J = 5.8 Hz, 1 H, O═CNH), 4.39 (m, 1 H, OCH), 4.29 (dd, J = 11.4, 2.1 Hz, 1 H, OCH_{2a}), 4.05 (dd, J = 11.4, 7.0 Hz, 1 H, OCH_{2b}), 3.51 (quart, J = 6.7 Hz, 2 H, O═CNCH$_2$), 2.98 (dd, J = 12.6, 7.1 Hz, 1 H, $OCCH_{2a}N$), 2.88 (dd, J = 12.6, 4.7 Hz, 1 H, $OCCH_{2b}N$), 2.71 (m, 6 H, NCH_2 and $PhCH_2$'s), 1.95 (quint, J = 7.3 Hz, 2 H, O═CNCCH$_2$), 1.84 (quint, J = 7.6 Hz, 2 H, CH_2). Anal. ($C_{30}H_{33}N_3O_3 \cdot HCl \cdot {}^1/_2CH_3OH$) C, H, N.

Binding Assay. IC_{50} values were estimated from a nonlinear single site fit to data obtained from competition binding experiments employing 10 drug concentrations run in duplicate. Radioligands used were [^{3}H]U-86170 (86.1 Ci/mmol, 1.7 nM) for D2-dopamine, [^{3}H]8-OH-DPAT (164.5 Ci/mmol, 1 nM) for 5-HT_{1A}, and [^{3}H]ketanserin (60 Ci/mmol, 1 nM) for 5-HT_2 receptors. In these assays the receptors were from mammalian clones expressed in CHO cell membranes.[19] Membranes for opioid and α_1-adrenergic binding were prepared from rat whole brain. Here the radioligands were [^{3}H]etorphine (33.2 Ci/mmol, 0.2 nM) and [^{3}H]prazosin (18.1 Ci/mmol, 0.9 nM), respectively. K_i values were calculated with the Cheng and Prusoff equation.[20] The data are inhibition constants in nanomolar (±SEM).

Mouse Hypothermia. Animals used in this hypothermia assay were Upjohn or Charles River CF-1 male mice weighing 18–22 g. Mice were divided into groups of four per dose and individually caged in 7 × 11 × 5 in. divided clear plastic cages with sawdust bedding and perforated metal tops a minimum of minutes prior to testing. After control rectal temperatures were measured in degrees Fahrenheit using a YSI Telethermometer, animals received a 0.1 mL sc injection of test compound. Twenty minutes later, rectal temperatures were again measured. A decrease of 2 °F or more was regarded as a positive hypothermic response. Drug doses started at 30 mg/kg and were decreased by half-log increments until zero out of four mice showed a positive hypothermic response. ED_{50}'s were determined by Spearman–Karber method for ED_{50}'s.[21] Oral-dosing experiments were similar to those for sc dosing except the drug was injected po with a rounded oral 18 gauge hypodermic syringe and the volume adjusted to 0.2 mL. Regardless of the route of administration, the mean maximum temperature drop recorded at 20 min following

(19) Chio, C. L.; Hess, G. F.; Graham, R. S.; Huff, R. M. A second molecular form of D_2 dopamine receptor in rat and bovine caudate nucleus. *Nature* **1990**, *343*, 266–269. Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT_{1A} receptor. *Nature* **1988**, *335*, 358–360. Pritchett, D. B.; Bach, A. W. J.; Wozny, M.; Taleb, O.; Toso, R. D.; Shih, J. C.; Seeburg, P. H. Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *EMBO J.* **1988**, *7*, 4135–4140.

(20) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(21) Finney, D. J. *Statistical Methods in Biological Research*; Haffner: New York, 1952; p 524.

any dose was noted as a crude index of drug efficacy and indirect measure of intrinsic activity.

Cardiovascular Evaluation in the Cat. Six mongrel cats (2.5–4.0 kg) were anesthetized with an intravenous injection of chloralose (80 mg/kg) and placed in a stereotaxic apparatus. The femoral artery and vein were cannulated in order to monitor arterial blood pressure and for intravenous drug injections, respectively. Heart rate (HR) was triggered from the EKG. A glass trachea tube was inserted and rectal temperature was maintained using a heating pad and/or lamp. Animals were allowed to breath spontaneously. Sympathetic nerve discharge (SND) was recorded under mineral oil from the isolated left inferior cardiac nerve using a bipolar platinum electrode with capacity coupled preamplification at low and high frequency half-amplitude responses at 1 and 500 Hz, respectively. SND was quantitated using cumulative integration (i.e. summation of voltage contained in sympathetic slow waves).

Mean arterial blood pressure (MAP), HR, and SND were allowed to equilibrate for 1 h after surgery. Pretreatment values for MAP, HR, and SND were averaged over the last 10 min of the equilibration period. A cumulative dose–response curve was constructed to determine the effects of U-86192A on MAP, HR, and SND. The starting dose of U-86192A was 0.01 mg/kg iv, and the dose of the drug was increased every 20 min to achieve total cumulative dose of 0.03, 0.1, 0.3 and 1.0 mg/kg iv. The effects of U-86192A on MAP, HR, and SND were determined 15–20 min after each dose. Following the last dose of U-86192A, the 5-HT_{1A} antagonist spiperone was administered intravenously at a dose of 1 mg/kg.

Acknowledgment. We thank Gurnam S. Gill and Phil G. Zaworski (Molecular Biology Research, Upjohn Laboratories) for providing membranes of 5-HT_2 receptor expressing clones for the binding studies.

Adenosine A_1 Antagonists. 2.† Structure–Activity Relationships on Diuretic Activities and Protective Effects against Acute Renal Failure

Fumio Suzuki,* Junichi Shimada, Hideaki Mizumoto, Akira Karasawa, Kazuhiro Kubo, Hiromi Nonaka, Akio Ishii, and Takashi Kawakita

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken 411, Japan. *Received January 3, 1992*

Diuretic activities of xanthine or nonxanthine adenosine antagonists and their ameliorative effects against glycerol-induced acute renal failure in rats were investigated in order to clarify the physiological and pathological function of adenosine receptors in the kidney. Diuretic and natriuretic activities of a variety of adenosine antagonists clarified systematically for the first time that the blockade of A_1 receptors is more important than that of A_2 receptors in sodium and water excretion and support the hypothesis that endogenous intrarenal levels of adenosine directly enhance tubular sodium readsorption. Studies of structure–activity relationships of 8-substituted xanthines in the acute renal failure demonstrated that the activation of adenosine A_1 receptor was an important factor in developing such a renal failure. A series of 8-(3-noradamantyl)xanthines exhibited the extremely potent diuretic and natriuretic activities (**24**; 2.5 μg/kg, po, the ratio of urinary excretion value in treated rats to urinary excretion value in control rats = 1.69, the ratio of Na^+/K^+ in treated rats to Na^+/K^+ in control rats = 1.76) and potent ameliorative effects against glycerol-induced acute renal failure (**24**; 10 μg/kg, ip, 55% inhibition). From our detailed studies of structure–activity relationships, we can speculate that some tissue differences of the adenosine A_1 receptor might exist between kidney and brain and sites of action for adenosine antagonists could be different between two renal pharmacological assays. 1,3-Dipropyl-8-(3-noradamantyl)xanthine, KW-3902 (**24**), was chosen for further studies and is under development as a drug for treating the acute renal failure.

Introduction

The relatively weak diuretic effects of xanthines such as caffeine (**1**) or theophylline (**2**) has been described for more than century.[1] Recently, the pharmacological basis for these diuretic actions has been proposed to be adenosine receptor antagonism.[2] In fact, exogenous adenosine produces intense antidiuretic and antinatriuretic effects in many species,[3] and these effects are competitively antagonized by theophylline[4] and mimicked by several adenosine analogues.[5]

Adenosine receptors are subdivided into subtypes, designated as A_1 and A_2.[6,7] A_1 receptors exhibit relatively high affinity to adenosine in binding studies (nM) and some are coupled to and inhibit adenylate cyclase. In contrast, A_2 receptors exhibit at lower affinity to adenosine (μM) and some are coupled to, but stimulate, adenylate cyclase.[8] Adenosine receptors, when activated, can elicit either vasoconstriction (A_1) or vasodilation (A_2). This biphasic action of exogenous adenosine has created uncertainty over the specific role of endogenous adenosine in the control of renal function. In the isolated kidney, perfused at constant pressure, stimulation of A_1 receptors

† Part 1 in the series of Adenosine A_1 Antagonist is ref 33c.

(1) Fredholm, B. B. Cardiovascular and Renal Actions of Methyl-xanthines. *Prog. Clin. Biol. Res.* **1984**, *158*, 303–330.
(2) Persson, C. G. A.; Erjefält, I.; Edholm, L. E.; Karlsson, J. A.; Lamm, C. J. Tracheal Relaxant and Cardiostimulant Actions of Xanthines can be Differentiated from Diuretic and CNS-Stimulant Effects. Role of Adenosine Antagonism? *Life Sci.* **1982**, *31*, 2673–2681.
(3) (a) Osswald, H.; Schmitz, H.-J.; Heindenreich, O. Adenosine Response of the Rat Kidney after Saline Loading, Sodium Restriction and Hemorrhagia. *Pfluegers Arch.* **1975**, *357*, 323–333. (b) Osswald, H.; Schmitz, H.-J.; Kemper, R. Renal Action of Adenosine: Effect on Renin Secretion in the Rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1978**, *303*, 95–99. (c) Osswald, H.; Spielman, W. S.; Knox, F. G. Mechanism of Adenosine-Mediated Decreases in Glomerular Filtration Rate in Dogs. *Cir. Res.* **1978**, *43*, 465–469.
(4) Osswald, H. Renal Effects of Adenosine and Their Inhibition by Theophylline in Dogs. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1975**, *288*, 79–86.
(5) Churchill, P. C.; Bidani, A. Renal Effects of Selective Adenosine Receptor Agonists in Anesthetized Rats. *Am. J. Physiol.* **1987**, *252*, F299–F303.

0022-2623/92/1835-3066$03.00/0